A RAPD Marker for the Are Anthracnose Resistance Gene in Beans

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Colletotrichum lindemuthianum (Sacc. et. Magn.) Scrib., the causal agent of the bean anthracnose is a serious seedborne fungus, capable of inducing complete yield losses on susceptible genotypes. The dominant gene 'Are', which confers resistance to Alpha, Beta, Gamma, Delta, Epsilon, and Lambda races of the fungus, was first found in a black bean from Venezuela, Cornell 49-242 (Mastenbroek, 1960), and has been widely used in North America and Europe as the main source of resistance against anthracnose. A potential risk of a resistance break-down exists, due to an over-reliance on such a narrow genetic base and the pathogenic variability of this fungus.
The use of molecular markers linked to major disease resistance genes in *Phaseolus vulgaris*, would provide breeders an efficient and rapid tool to screen for desirable individuals. This would facilitate the introgression of multilple resistance genes into a single cultivar by overcoming potential genetic difficulties due to epistatic interactions. Hence, the implementetaion of a gene-marker would make feasible the stabilization of genetic resistance.
The work presented here, describes the strategy followed in developing a Random Amplified Polymorphic DNA (RAPD) marker linked to the bean anthracnose resistant Are gene.

A pedigree generated (F_{11}) inbred line K86002 showing heterogeneity for the Are gene was utilized as a source of near isogenic lines (NILs). A bulked segregant analysis approach (Michelmore et al. 1991) was implemented to assist in the identification of RAPD markers associated with the target locus. Two DNA bulks were formed by using four homozygote resistant (R) and four homozygote susceptible (S) F_2 individuals derived from a cross between two navy NILs heterogeneous for the Are locus. Navy bulks were designated A4512 R and A4512 S respectively. A second pair of navy inbred (F_6) lines (N85006) heterogeneous for the A locus were included as an alternative check to assist in the detection of false positive polymorphisms .The three DNA pairs of genetic materials were screened with random decamer primers to identify RAPD marker linked to the Are locus. Progeny of 108 F_2 individuals from the K86002 (SxR) cross was used as the mapping population to confirm the presence and assess the degree of linkage between the RAPD marker and the Are allele.

All pathogen inoculations were done using a suspension of 1.2×10^{-6} spores ml^{-1}. Inoculated plants were placed in a controlled dew chamber for 48 hours, and then transferred to greenhouse conditions. Disease ratings were made 7 days post-inoculation. Progeny test of 18 plants from each K86002 $F_{2:3}$ line were carried out to confirm the disease expression of F_2 plants and determine their actual genotype.

A simple and quick DNA extraction method developed in our laboratory, and previously described (Afanador et al. 1993) was adopted. The Polymerase Chain Reaction (PCR) protocol used consisted of 3 cycles of 1 min/94°C, 1 /35°C, 2 min/72°C; 34 cycles of 10 s/94°C, 20 s/40°C, 2 min/72°C; 1 cycle of 5 min/72°C; 1 s "Auto-segment Extension" (for extension phase of 34-cycle portion of PCR). Amplification was carried out in a Perkin Elmer Cetus DNA Thermal Cycler 480. Description of the PCR reactions have been previously reported (Haley et al. 1993). Approximately 10 ng of genomic DNA template and 10 ng of a decamer primer (Operon Technologies, Alameda, Calif.) were performed in a 18.8 ul reaction, containing 2 units of Stoffel Fragment Polymerase (Perkin Elmer CETUS, Norwalk, Conn.). Amplified DNA Were resolved by electrophoresis in 1.4% agarose gel.

A total of 346 primers were screened against the 3 DNA pair combinations of resistant and susceptible genotypes from Andean and Mesoamerican origin. Five putatively linked RAPD markers to the Are locus were observed, but only one of these RAPDs, designated OQ41440 generated by a 5'-AGTGCGCTGA-3' decamer, was found to be closely linked (2.0 ± 1.4cM) in coupling with the resistance Are allele. Monogenic inheritance of the Are and OQ41440 loci was confirmed by the Chi Squared test (X^2=96.01, P=0.00) performed on a F_2 population of 108 segregating individuals from the Andean K86002 (S) x K86002 (R) cross using Linkage 1 (Suiter et al.1983).

Considering that the Are gene was originally introgressed from a Mesoamerican bean genotype into the Andean kidney bean germplasm, recombination suppression due to linkage drag in the foreign genetic background could be expected. This may result in an observed tighter linkage between a DNA marker and the locus of interest. Therefore, the overall usefulness of OQ41440 RAPD marker across *Phaseolus* gene pools requires further evaluation. The use of OQ41440 for marker-based selection will afford the opportunity of pyramiding anthracnose resistant genes, as other epistatic resistance genes are characterized, and new RAPDs are identified.

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